



**UNIVERSITI PUTRA MALAYSIA**

**TOXICITY OF ANTIFUNGAL DRUGS ITRACONAZOLE AND  
FLUCONAZOLE IN RATS**

**NOR SHAHIDA ABDUL RAHMAN**

**FPSK(M) 2004 8**

**TOXICITY OF ANTIFUNGAL DRUGS ITRACONAZOLE AND  
FLUCONAZOLE IN RATS**

**By**

**NOR SHAHIDA ABDUL RAHMAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in  
Fulfilment of the Requirements for the Degree of Master of Science**

**March 2004**



## DEDICATION

“Dedicated especially to my parents Abdul Rahman Mat and Tuan Zaharah Tuan Yusoff, sisters, brothers and all those individuals behind the scenes who make me possible to complete my study successfully.”

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

## **TOXICITY OF ANTIFUNGAL DRUGS ITRACONAZOLE AND FLUCONAZOLE IN RATS**

By

**NOR SHAHIDA ABDUL RAHMAN**

**March 2004**

**Chairman: Associate Professor Muhammad Nazrul Hakim Abdullah, Ph.D.**

**Faculty: Medicine and Health Sciences**

Itraconazole and Fluconazole are the newer antifungal drugs that have been used for several years. Both these drugs have a broad-spectrum antifungal activity and currently are used to treat infections caused by *Candida albicans*, *Aspergillus spp.*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Histoplasma capsulatum*, *Cryptococcus neoformans* and many others. The objective of this study is to investigate the *in vitro* and *in vivo* cytotoxicity of these two antifungal drugs. The *in vitro* and *in vivo* cytotoxicity of fluconazole and itraconazole were studied in thirty eight male Sprague Dawley rats. Freshly isolated rat hepatocytes were obtained for the *in vitro* treatment of fluconazole and itraconazole using a liver perfusion technique. The cell viability test was done by trypan blue exclusion. As a result, both fluconazole and itraconazole cause a reduction in cell viability of hepatocytes. However, itraconazole exerted its cytotoxicity more than fluconazole in both time- and dose-dependent manner. Meanwhile, cytotoxicity of itraconazole was reduced significantly by Phenobarbital pretreatment. Phenobarbital did not have any effect on the

cytotoxicity induced by fluconazole. *In vivo* studies revealed that rat's liver and kidney treated with repeated-doses of itraconazole showed a significantly higher in total protein in liver and kidney and significant increase in serum ALP and ALT activity. This is in agreement with histological findings that the rat treated with repeated-doses of itraconazole showed severe histological features compared to fluconazole. Morphological changes such as inflammation and fibrosis of liver were frequently seen in repeated-doses of itraconazole. This present study suggests that Phenobarbital plays a role in the cytoprotection of hepatocytes to itraconazole-induced but not fluconazole-induced cytotoxicity *in vitro*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KETOKSIKAN DADAH ANTI-KULAT ITRACONAZOLE DAN  
FLUCONAZOLE DALAM TIKUS**

Oleh

**NOR SHAHIDA ABDUL RAHMAN**

**Mac 2004**

**Pengerusi: Professor Madya Muhammad Nazrul Hakim Abdullah, Ph.D.**

**Fakulti: Perubatan dan Sains Kesihatan**

Itraconazole dan fluconazole tergolong di antara dadah anti-kulat yang baru diperkenalkan dan telah digunakan sejak beberapa tahun yang lalu. Kedua-dua dadah anti-kulat ini telah digunakan secara meluas terutamanya dalam merawat jangkitan yang disebabkan oleh *Candida albicans*, *Aspergillus spp.*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Histoplasma capsulatum*, *Cryptococcus neoformans* dan sebagainya. Objektif kajian ini adalah untuk mengetahui ketoksikan kedua-dua dadah ini secara *in vitro* dan *in vivo*. Di dalam kajian ini tiga puluh lapan ekor tikus jantan dari spesies Sprague Dawley telah digunakan. Pengasingan sel hepatosit tikus segar dapat dibuat untuk digunakan dalam rawatan *in vitro* oleh fluconazole dan itraconazole dengan menggunakan teknik perfusi hati. Ujian untuk menguji sel-sel hidup dibuat menggunakan tripan biru. Fluconazole dan itraconazole menyebabkan penurunan kepada bilangan sel hepatosit yang hidup. Walaubagaimanapun, itraconazole menunjukkan kesan ketoksikan yang lebih tinggi berbanding fluconazole dalam keadaan berkadar langsung dengan masa dan dos.

Sementara itu, ketoksikan yang disebabkan oleh dadah anti-kulat itraconazole dapat dikurangkan dengan menggunakan Phenobarbital. Phenobarbital tidak mempengaruhi ketoksikan yang disebabkan oleh fluconazole. Kajian *in vivo* juga menunjukkan terdapat peningkatan dalam jumlah protein dan aktiviti serum ALP dan ALT di dalam hati dan buah pinggang tikus yang diberi suntikan itraconazole secara berulang-kali. Secara histologinya, tikus yang diberi suntikan itraconazole secara berulang-kali menunjukkan kesan ketoksikan yang ketara terhadap hati dan buah pinggang tikus jika dibandingkan dengan tikus yang diberi suntikan fluconazole secara berulang-kali. Perubahan morfologi seperti keradangan dan fibrosis pada hati telah dilihat dengan kerap dalam suntikan itraconazole secara berulang-kali. Dengan itu, kajian ini telah menunjukkan bahawa phenobarbital memainkan peranan dalam mengurangkan ketoksikan yang dihasilkan oleh itraconazole tetapi tidak mempengaruhi ketoksikan yang disebabkan oleh fluconazole secara *in vitro*.

## **ACKNOWLEDGEMENTS**

**In the name of Allah the Most Gracious and Merciful**

Without exceptions, each of the illustrations was specially selected and prepared for this project paper, whilst accepting full responsibility for the entire contents; I am indebted to many individuals who have made invaluable contributions in their specialized fields.

First and foremost, I would like to express my deepest and most sincere gratitude to my chairman of supervisory committee, Associate Professor Dr. Muhammad Nazrul Hakim Abdullah whose expert guidance, advice and support has helped me to complete this research. His kindness, affection encouragement and moral support gave me the courage and ability to overcome all the problems, which I have faced from time to time during the course of my work.

I am also indebted to members of my supervisory committee and I wish to express my deepest thanks to Prof. Dato' Dr. Abdul Salam Abdullah, Associate Professor Dr. Fauziah Othman and Dr. Wan Nordin Wan Mahmud for their interest and invaluable suggestions that had enable me to carry on with my project successfully.

I really appreciate Dr. Mohd Khairi Hussein and Puan Hasiah Abdul Hamid for their helpful and guidance throughout my project. A special mention must be given to Associate Professor Dr. Zarida Hambali for letting me used the facilities and materials





of her laboratory. I would also like to express my heartfelt appreciation to Faizah Sanat (my best friend and also partner in research), who was and still by my side during the trials and tribulation during the course of my work.

I also would like to express my sincere gratitude to all the staff: Puan Sapiyah from the Histology Laboratory, Faculty of Veterinary Medicine; Puan Siti Muskinah, Puan Juita, Puan Normah, En. Rijalana and En. Ramli from the Faculty of Medicine and Health Sciences; Mr. Ho Oi Kuen from the Institute of Bioscience and En. Kufli from the Physiology Laboratory, Faculty of Veterinary Medicine for their co-operation.

Last but not least to my entire colleague, Hazliana, Rohayu Izanwati, Elysha, Azmahani, Solihah, Reezal, Ahmad Saifuddin and Yunus for their willingness to help me when I needed it most. Finally, I wish to extend my appreciation to all who are involved either directly or indirectly in this research.

I certify that an Examination Committee met on 5<sup>th</sup> March 2004 to conduct the final examination of Nor Shahida Abdul Rahman on her Master of Science thesis entitled “Toxicity of Antifungal Drugs Itraconazole and Fluconazole in Rats” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Patimah Ismail, B. Sc., Ph.D.**

Associate Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Chairman)

**Muhammad Nazrul Hakim Abdullah, D.V.M., Ph.D.**

Associate Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Member)

**Dato’ Abdul Salam Abdullah, Ph.D.**

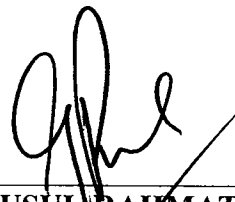
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Fauziah Othman, D.V.M., M.Sc., Ph.D.**

Associate Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Member)

**Wan Nordin Wan Mahmud, D.V.M., M.V.S.**

Faculty of Veterinary Medicine  
Univesiti Putra Malaysia  
(Member)



**GULAM RUSUL RAHMAT ALI, Ph.D.**  
Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 20 APR 2004

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

**Muhammad Nazrul Hakim Abdullah, D.V.M., Ph.D.**

Associate Professor  
Department of Biomedical Sciences  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Dato' Abdul Salam Abdullah, Ph.D.**

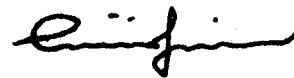
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Fauziah Othman, D.V.M., M.Sc., Ph.D.**

Associate Professor  
Department of Biomedical Sciences  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

**Wan Nordin Wan Mahmood, D.V.M., M.V.S.**

Lecturer  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)



---

**AINI IDERIS, Ph.D.**

Professor/ Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 17 MAY 2004

## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



---

**NOR SHAHIDA ABDUL RAHMAN**

Date: 13 APR 2004

## TABLES OF CONTENTS

	<b>Page</b>
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxvi
 <b>CHAPTER</b>	
1 INTRODUCTION	1
2 LITERATURE REVIEW	5
2.1 Fluconazole	5
2.1.1 Mode of Action	6
2.1.2 Pharmacokinetics	7
2.1.3 Clinical Efficacy	8
2.1.4 Adverse Effects	9
2.2 Itraconazole	10
2.2.1 Mode of Action	11
2.2.2 Pharmacokinetics	13
2.2.3 Clinical Efficacy	14
2.2.4 Adverse Effects	14
2.3 Liver	15
2.3.1 Normal Morphology of Liver	15
2.3.2 Biochemical Evaluation of Liver Functions	16
2.3.2.1 Plasma ALT	16
2.3.2.2 Plasma ALP	17
2.4 Drug Metabolism	17
2.4.1 Definition	17
2.5 Kidney	19
3 GENERAL MATERIALS AND METHODS	20
3.1 Experimental Animals	20
3.2 Record of Changes in Body Weight	20
3.3 Collection of Blood Samples	20
3.4 Collection of Tissue Samples	21
3.5 Lesion Scoring	21
3.5.1 Liver	22
3.5.2 Kidney	23
3.5.3 Stomach-Duodenum	24



3.6	Chemicals and Drugs	24
3.7	Methods	26
3.7.1	Liver Perfusion	26
3.7.2	Measurement of Cell Viability	28
3.7.3	Preparation of Supernatant	29
3.7.4	Protein Determination in Tissue Homogenate	29
3.7.5	Biochemical Evaluation of liver Functions	31
3.7.5.1	Plasma ALT	31
3.7.5.2	Plasma ALP	31
4	<i>IN VITRO</i> CYTOTOXICITY OF ITRACONAZOLE AND FLUCONAZOLE IN NORMAL RAT HEPATOCYTES	33
4.1	Introduction	33
4.2	Objective	34
4.3	Material and Methods	34
4.3.1	Experimental Animals	34
4.3.2	Isolation of Hepatocytes	35
4.3.3	Treatment of Hepatocytes Suspension	35
4.3.4	Statistical Analysis	35
4.4	Result	36
4.4.1	Perfusion Process	36
4.4.2	Trypan Blue Exclusion Test	36
4.4.2.1	Treatment of Fluconazole in Determining Cells Viability	36
4.4.2.2	Treatment of Itraconazole in Determining Cells Viability	39
4.4.2.3	The Comparison of the Effect of Fluconazole and Itraconazole on Un-pretreated Hepatocytes	42
4.5	Discussion	52
5	<i>IN VITRO</i> CYTOTOXICITY OF ITRACONAZOLE AND FLUCONAZOLE IN PHENOBARBITAL-INDUCED RAT HEPATOCYTES	55
5.1	Introduction	55
5.2	Objective	56
5.3	Material and Methods	56
5.3.1	Experimental Animals	56
5.3.2	Isolation of Hepatocytes	57
5.3.3	Treatment of Hepatocytes Suspension	57
5.3.4	Statistical Analysis	57
5.4	Result	57
5.4.1	Phenobarbital Pretreatment	57
5.4.2	Trypan Blue Exclusion Test	58
5.4.2.1	Treatment of Fluconazole on PB-induced Rat Hepatocytes in Determining Cells Viability	58
5.4.2.2	Treatment of Itraconazole on PB-induced	61

	Rat Hepatocytes in Determining Cells Viability	
	5.4.2.3 Comparison Between PB-induced and Normal Rat Hepatocytes after Treatment with Fluconazole	65
	5.4.2.4 Comparison Between PB-induced and Normal Rat Hepatocytes after Treatment with Itraconazole	74
	5.4.2.5 The Comparison on the Effects of Fluconazole and Itraconazole on PB-induced Rat Hepatocytes	83
5.5	Discussion	93
6	<i>IN VIVO</i> CYTOTOXICITY OF ITRACONAZOLE AND FLUCONAZOLE	96
6.1	Introduction	96
6.2	Objective	97
6.3	Material and Methods	97
	6.3.1 Experimental Animals	97
	6.3.2 Collection of Blood Samples	97
	6.3.3 Protein Assay	98
	6.3.4 Liver Function Test	98
	6.3.5 Histology	98
	6.3.5.1 Light Microscopy	98
	6.3.5.2 Lesion Scoring	99
	6.3.6 Statistical Analysis	99
	6.3.7 Results	100
	6.3.7.1 Effects of the Acute and Chronic Dose of Fluconazole and Itraconazole Treatment in Rat	100
	6.3.7.2 Changes in Body Weight	100
	6.3.7.3 Liver and Kidney Weight after Acute and Chronic Dosing with Fluconazole and Itraconazole	101
	6.3.7.4 Total Protein in Liver and Kidney after Acute and chronic Dosing with Fluconazole and Itraconazole	101
	6.3.7.5 Activities of Serum ALP and ALT before and after treatment with Acute and Chronic Doses of Fluconazole and Itraconazole	111
	6.3.7.6 Morphology Study of Liver, Kidney, Stomach and Duodenum of Rats Treated with Single and Repeated Doses of Fluconazole and Itraconazole	120
	6.3.8 Discussion	137
7	GENERAL DISCUSSION AND CONCLUSION	141
8	RECOMMENDATION	143

BIBLIOGRAPHY	145
APPENDICES	157
BIODATA OF THE AUTHOR	170





## LIST OF TABLES

Table	Page
2.1 Most common clinical adverse effects in 4,048 subjects receiving fluconazole	10
4.1 Time-dependent viability of rat hepatocytes during incubation with various concentrations of fluconazole at different time points	37
4.2 Dose-dependent viability of rat hepatocytes during incubation with various concentrations of fluconazole at different time points	38
4.3 Time-dependent viability of rat hepatocytes during incubation with various concentrations of itraconazole at different time points	40
4.4 Dose-dependent viability of rat hepatocytes during incubation with various concentrations of itraconazole at different time points	41
5.1 Time-dependent viability of PB-induced rat hepatocytes during incubation with various concentrations of fluconazole at different time points	59
5.2 Dose-dependent viability of PB-induced rat hepatocytes during incubation with various concentrations of fluconazole at different time points	60
5.3 Time-dependent viability of PB-induced rat hepatocytes during incubation with various concentrations of itraconazole at different time points	63
5.4 Dose-dependent viability of PB-induced rat hepatocytes during incubation with various concentrations of itraconazole at different time points	64
6.1 Effects of single-doses treatment of fluconazole and itraconazole on liver and kidney weight of rat	102
6.2 Effects of repeated-doses treatment of fluconazole and itraconazole on liver and kidney weight of rat	103
6.3 Effect of itraconazole on samples for acute group at different doses	125
6.4 Effect of fluconazole on samples for acute group at different doses	125
6.5 Effect of fluconazole on samples of chronic group at different doses	126



6.6	Effect of itraconazole on samples of chronic group at different doses	126
6.7	Effects of fluconazole and itraconazole on samples for the acute group	127
6.8	Effects of fluconazole and itraconazole on samples for the acute group	127
6.9	Effects of fluconazole and itraconazole on samples for the chronic group	128
6.10	Effects of fluconazole and itraconazole on samples for the chronic group	128



## LIST OF FIGURES

Figure	Page
1.1 Chemical structure of fluconazole and itraconazole	2
4.1 Time-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 0mM	44
4.2 Time-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 0.0001mM	44
4.3 Time-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 0.001mM	45
4.4 Time-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 0.01mM	45
4.5 Time-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 0.1mM	46
4.6 Time-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 1.0mM	46
4.7 Dose-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 0.25hr.	47
4.8 Dose-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 0.5hr.	47
4.9 Dose-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 0.75hr.	48
4.10 Dose-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 1hr.	48
4.11 Dose-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 2hr.	49
4.12 Dose-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 3hr.	49
4.13 Dose-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 4hr.	50

4.14	Dose-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 5hr.	50
4.15	Dose-dependent cytotoxicity of fluconazole and itraconazole on un-pretreated rat hepatocytes at 6hr.	51
5.1	Time-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 0mM	66
5.2	Time-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 0.0001mM	66
5.3	Time-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 0.001mM	67
5.4	Time-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 0.01mM	67
5.5	Time-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 0.1mM.	68
5.6	Time-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 1.0mM	68
5.7	Dose-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 0.25hr.	69
5.8	Dose-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 0.5hr.	69
5.9	Dose-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 0.75hr.	70
5.10	Dose-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 1hr.	70
5.11	Dose-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 2hr.	71
5.12	Dose-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 3hr.	71
5.13	Dose-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 4hr.	72
5.14	Dose-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 5hr.	72

5.15	Dose-dependent cytotoxicity of fluconazole on PB-induced and normal rat hepatocytes at 6hr.	73
5.16	Time-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 0mM	75
5.17	Time-dependent cytotoxicity of itraconazole on PB-induced and Normal rat hepatocytes at 0.0001mM	75
5.18	Time-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 0.001mM	76
5.19	Time-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 0.01mM	76
5.20	Time-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 0.1mM	77
5.21	Time-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 1.0mM	77
5.22	Dose-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 0.25hr.	78
5.23	Dose-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 0.5hr.	78
5.24	Dose-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 0.75hr.	79
5.25	Dose-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 1hr.	79
5.26	Dose-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 2hr.	80
5.27	Dose-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 3hr.	80
5.28	Dose-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 4hr.	81
5.29	Dose-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 5hr.	81
5.30	Dose-dependent cytotoxicity of itraconazole on PB-induced and normal rat hepatocytes at 6hr.	82

5.31	Time-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 0mM	85
5.32	Time-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 0.0001mM	85
5.33	Time-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 0.001mM	86
5.34	Time-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 0.01mM	86
5.35	Time-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 0.1mM	87
5.36	Time-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 1.0mM	87
5.37	Dose-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 0.25hr.	88
5.38	Dose-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 0.5hr.	88
5.39	Dose-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 0.75hr.	89
5.40	Dose-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 1hr.	89
5.41	Dose-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 2hr.	90
5.42	Dose-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 3hr.	90
5.43	Dose-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 4hr.	91
5.44	Dose-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 5hr.	91
5.45	Dose-dependent cytotoxicity of fluconazole and itraconazole on PB-induced rat hepatocytes at 6hr.	92
6.1	Mean body weight of rats treated with repeated-doses of fluconazole	100

6.2	Mean body weight of rats treated with repeated-doses of itraconazole	101
6.3	The mean total protein in liver of rats treated with single-doses of fluconazole	105
6.4	The mean total protein in liver of rats treated with single-doses of itraconazole	105
6.5	The mean total protein in liver of rats treated with repeated-doses of fluconazole	106
6.6	The mean total protein in liver of rats treated with repeated-doses of itraconazole	106
6.7	The mean total protein in kidney of rats treated with single-doses of fluconazole	107
6.8	The mean total protein in kidney of rats treated with single-doses of itraconazole	107
6.9	The mean total protein in kidney of rats treated with repeated-doses of fluconazole	108
6.10	The mean total protein in kidney of rats treated with repeated-doses of itraconazole	108
6.11	The comparison of the mean total protein in liver of rats treated with single-doses of fluconazole and itraconazole	109
6.12	The comparison of the mean total protein in liver of rats treated with repeated-doses of fluconazole and itraconazole	109
6.13	The comparison of the mean total protein in kidney of rats treated with single-doses of fluconazole and itraconazole	110
6.14	The comparison of the mean total protein in kidney of rats treated with repeated-doses of fluconazole and itraconazole	110
6.15	The mean plasma ALT levels in rats treated with single-doses of fluconazole	112
6.16	The mean plasma ALT levels in rats treated with single-doses of itraconazole	112
6.17	The mean plasma ALP levels in rats treated with single-doses of fluconazole	113

6.18	The mean plasma ALP levels in rats treated with single-doses of itraconazole	113
6.19	The mean plasma ALT levels in rats treated with repeated-doses of fluconazole	114
6.20	The mean plasma ALT levels in rats treated with repeated-doses of itraconazole	114
6.21	The mean plasma ALP levels in rats treated with repeated-doses of fluconazole	115
6.22	The mean plasma ALP levels in rats treated with repeated-doses of Itraconazole	115
6.23	The mean plasma ALT levels of pre-treated rats with single-doses of fluconazole and itraconazole	116
6.24	The mean plasma ALT levels of post-treated rats with single-doses of fluconazole and itraconazole	116
6.25	The mean plasma ALP levels of pre-treated rats with single-doses of fluconazole and itraconazole	117
6.26	The mean plasma ALP levels of post-treated rats with single-doses of fluconazole and itraconazole	117
6.27	The mean plasma ALT levels of pre-treated rats with repeated-doses of fluconazole and itraconazole	118
6.28	The mean plasma ALT levels of post-treated rats with repeated-doses of fluconazole and itraconazole	118
6.29	The mean plasma ALP levels of pre-treated rats with repeated-doses of fluconazole and itraconazole	119
6.30	The mean plasma ALP levels of post-treated rats with repeated-doses of fluconazole and itraconazole	119
6.31	Light micrograph of liver from normal non-treated rat	129
6.32	Light micrograph of rat's liver treated with repeated high dose of 100mg/kg fluconazole	129
6.33	Light micrograph of rat's liver treated with repeated high dose of 100mg/kg fluconazole	130



6.34	Light micrograph of rat's liver treated with repeated high dose of 100mg/kg itraconazole	130
6.35	Light micrograph of rat's liver treated with repeated high dose of 100mg/kg itraconazole	131
6.36	Light micrograph of rat's liver treated with repeated high dose of 100mg/kg itraconazole	131
6.37	Light micrograph of rat's liver treated with repeated high dose of 100mg/kg itraconazole	132
6.38	Light micrograph of kidney from normal non-treated rat	133
6.39	Light micrograph of rat's kidney treated with single dose of itraconazole	133
6.40	Light micrograph of rat's kidney treated with single dose of itraconazole	134
6.41	Light micrograph of rat's kidney treated with repeated high dose of itraconazole	134
6.42	Light micrograph of stomach from normal non-treated rat	135
6.43	Light micrograph of stomach from normal non-treated rat	135
6.44	Light micrograph of rat's stomach treated with repeated high dose of 100mg/kg itraconazole	136
6.45	Light micrograph of duodenum from normal non-treated rat	136
A	Perfusion buffer I	157
A	Perfusion buffer II	157
B	Complete medium	158
C	Tissue dehydration in the histokinette	159
D	Staining process (Hematoxylin and Eosin)	160
E	Effect of chronic and acute fluconazole treatment on total protein in liver and kidney of rat	161
F	Effect of chronic and acute itraconazole treatment on total protein in liver and kidney of rat	162